Plant and Soil **244:** 189–197, 2002.

Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi

Metabolism and transport in AM fungi

Berta Bago[1,5], Philip E. Pfeffer[2], Warren Zipfel[3], Peter Lammers[4] & Yair Shachar-Hill[4]
[1]*Estación Experimental del Zaidín (CSIC), Calle Profesor Albareda 1, 18008-Granada, Spain.*
[2]*Eastern Regional Research Center, ARS-USDA, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA.*
[3]*Cornell University, Ithaca, NY, USA.*
[4]*New Mexico State University, Las Cruces, NM 88003, USA.*
[5]*Corresponding author* (Present address: Centro de Investigaciones sobre Desertificación (CSIC/UV/GV), Camí de la Marjal s/n, 46470 Albal (Valencia), Spain)*

Key words: arbuscular mycorrhizal fungi, carbon metabolism, monoxenic cultures, multiphoton microscopy, nuclear magnetic resonance spectroscopy, nutrient translocation

Abstract

In the last few years the application of modern techniques to the study of arbuscular mycorrhizas has greatly increased our understanding of the mechanisms underlying carbon metabolism in these mutualistic symbioses. Arbuscular mycorrhizal (AM) monoxenic cultures, nuclear magnetic resonance spectroscopy together with isotopic labeling, and analyses of expressed sequence tags (ESTs) have shed light on the metabolic processes taking place in these interactions, particularly in the case of the mycobiont. More recently, *in vivo* multiphoton microscopy has provided us with some new insights in the allocation and translocation processes which play crucial roles in the distribution of host plant-derived C throughout the fungal colony. In this mini-review we highlight recent advances in these fields, with special attention to the visualization of oleosomes (i.e., lipid bodies) as they move along the long, coenocytic AM fungal hyphae. Volumetric measurements of such oleosomes have allowed us to estimate the flux of triacylglycerides from the intraradical to the extraradical phase of the AM fungal colony. We raise questions and postulate regulatory mechanisms for C metabolism and translocation within the arbuscular mycorrhizal fungal colony.

Transport processes in arbuscular mycorrhizal fungi

Nutrition in arbuscular mycorrhizas is based on the acquisition of soil nutrients by the fungus (George et al., 1995; Jakobsen, 1999; Koide and Schreiner, 1992), and fixation of atmospheric C by the plant (Ho and Trappe, 1973), and on the exchange of these nutrients at specially adapted symbiotic interfaces (Gianinazzi-Pearson et al., 1991; Smith and Read, 1997; Smith and Smith, 1990, 1997). Traditionally much attention has been focused on the mechanisms implied in these two transport processes, nutrient uptake and nutrient transfer (Figure 1; see Ferrol et al., 2002 and Burleigh and Bechman, 2002). In comparison, little is known about other two key classes of transport processes that take place within the arbuscular mycorrhizal (AM) fungus, both of them intracellular: the transport of nutrients into and out of the organelles where they are metabolized and/or stored, and the bidirectional translocation of nutrients (either soil- or plant-derived) along the coenocytic AM fungal hyphae (Figure 1).

Subcellular compartmentation of metabolism in the filamentous fungi is poorly understood, and translocation even less so. Most metabolic studies in fungi have been carried out in yeasts, unicellular organisms in which there is no bulk translocation. Moreover, in most fungal textbooks translocation in filamentous fungi (if at all considered) is usually treated in a very general manner and most often references are to higher

* FAX No: +34-96-127-0967. E-mail: berta.bago@uv.es

fungi, that have septa along their hyphae (e.g., Gow and Gadd, 1984; Jennings, 1995; but see Ashford and Allaway, this volume). Arbuscular mycorrhizal fungi by contrast have coenocytic (i.e., aseptate) multinuclear hyphae (Balestrini et al., 1992), which presents them and AM researchers with unusual challenges.

Carbon metabolism in AM fungi: an overview

The major fluxes of carbon change dramatically during the AM fungal life cycle. The intraradical phase, the extraradical phase and the germination phase each present distinctive characteristics (Bago et al., 2000). We will briefly review here C metabolism in intra- and extraradical hyphae, i.e., in the symbiotic fungal colony.

The intraradical mycelium (IRM) acquires hexose from the root and converts it into trehalose and glycogen

In vivo NMR coupled with ^{13}C-labeling demonstrated that hexose is taken up by the IRM and that trehalose and glycogen are the first substantial fungal C pool labeled from hexose which had been taken up by the intraradical fungal structures (Shachar-Hill et al., 1995). This contrasts with observations in the ectomycorrhizal symbiosis (Martin et al., 1985, 1998), where glucose is metabolized into these compounds via mannitol and where other carbohydrates also accumulate (Pfeffer et al., 1996, 2001). Glycogen and trehalose may serve to buffer cytoplasmic hexose levels (Bago et al., 2000). Recent labeling experiments show that glycogen is also translocated from IRM to ERM, particularly before sporulation (Figure 1; Bago et al., unpublished results). NMR spectroscopy studies, enzymatic determinations, radiorespirometry and molecular biology all indicate the functioning of glycolysis, the tricarboxylic acid (TCA) cycle, and the pentose phosphate pathway (PPP) in AM fungal intraradical structures (Harrier et al., 1998; MacDonald and Lewis 1978; Pfeffer et al., 1999; Saito, 1995; Solaiman and Saito, 1997). The results of recent experiments using ^{14}C-labeling in monoxenic cultures are also consistent with the activity of these pathways (Séjalon-Delmas et al., 2001).

Storage lipids are made in the intraradical mycelium

AM fungi are oleogenic fungi and store large amounts of lipid as triacylglycerides (TAGs) (Beilby, 1983; Beilby and Kidby, 1980; Cooper and Lösel, 1978; Gaspar et al., 1994, 1997; Jabaji-Hare, 1988; Nagy et al., 1980). The synthesis TAG is a substantial sink for carbon in the intraradical hyphae (Lösel and Cooper 1979; Pfeffer et al., 1999; Bago et al., 2000). ^{13}C-labeling and NMR studies indicate that the host-derived hexoses are metabolized by AM fungi (via glycolysis) to triose and acetyl CoA. Labeling patterns suggest that fatty acids are synthesized normally via acetyl CoA carboxylase and the FA synthase complex, then elongated and/or desaturated, and esterified with a glyceryl moiety via acyltransferases to constitute TAGs (Pfeffer et al., 1999; Bago et al., 2000).

The major fluxes of carbon in the IRM thus appear to be: efficient uptake of host-derived hexose, conversion to trehalose and glycogen as interim storage forms and the synthesis of large amounts of storage lipids (Figure 1).

The extraradical mycelium (ERM) is unable to acquire exogenous hexose or synthesize storage lipids

^{13}C-labeling and NMR spectroscopy have demonstrated that, unlike the intraradical fungal phase, extraradical fungal structures cannot absorb exogenous hexose (Pfeffer et al., 1999). Recent ^{14}C-labeling experiments (Séjalon-Delmas et al., 2001) support this view. Glycolytic enzyme activities in the ERM have been found to be low or absent (MacDonald and Lewis 1978; Saito, 1995; Séjalon-Delmas et al., 2001), whereas a substantial gluconeogenic flux fueled by the glyoxylate cycle does exist (Figure 1) (Lammers et al., 2001; Pfeffer et al., 1999). cDNA sequences for isocitrate lyase and malate synthase, the two key enzymes of glyoxylate cycle, have been characterized and their expression in the ERM demonstrated (Lammers et al., 2001). PPP activity also appears to be substantial in the extraradical hyphae (Figure 1), with both enzymatic activity measurements (Saito, 1995) and isotopic labeling data (Pfeffer et al., 1999) suggesting higher flux through this pathway than in the intraradical phase. All these data indicate that gluconeogenic C fluxes in the ERM are very substantial, and that this is mainly fuelled by the consumption of storage lipids (Figure 1). Thus the carbohydrate requirements of the ERM are met via TAG catabolism and gluconeogenesis, and some export of glycogen from the IRM.

Using $^{2}H_2O$-labeling Pfeffer and co-workers (1999) demonstrated that little or no storage lipid production occurred in AM extraradical fungal structures.

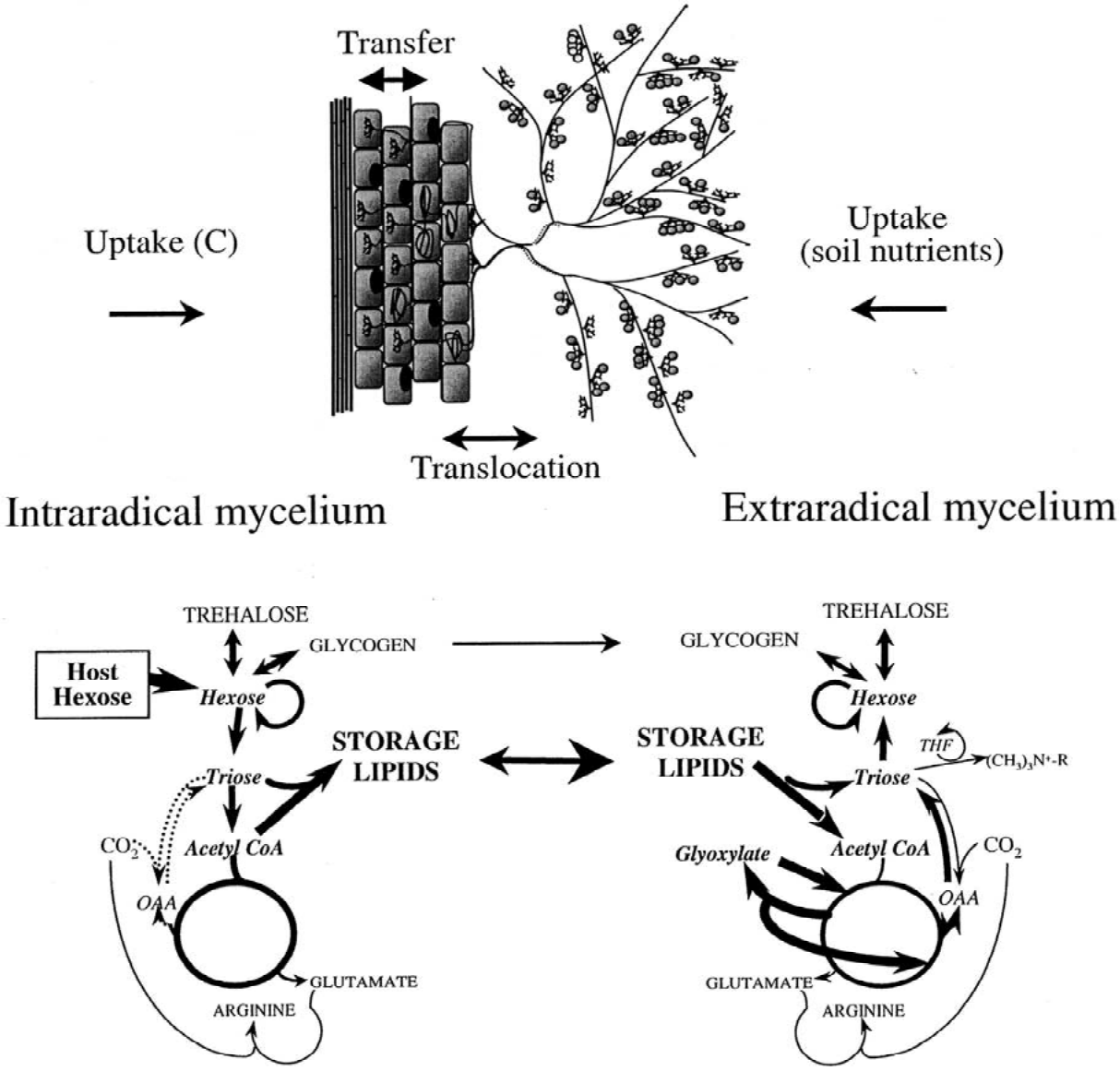

Figure 1. Transport processes occurring along the AM fungal colony (upper pannel) and C metabolic pathways and translocation events (lower pannel) taking place in and between the intraradical and extraradical fungal mycelium.

This has been supported by further experiments (Lammers et al., 2001) in which no label was detected in extraradical fatty acids after ^{13}C-labeled acetate (a direct intermediate in fatty acid synthesis) was supplied to the ERM. When ^{13}C-glycerol (a substrate for both FA and glycerol synthesis) was supplied, only the glyceryl moiety of the TAGs became labeled; again no label in the FA moieties was detected (Bago et al. unpublished results). Supplying any of several precursors to mycorrhizal roots resulted in high levels of labeling in the storage lipids of the ERM (Bago et al., 1999b; Lammers et al., 2001). Thus the lipids needed to sustain metabolism and growth of the ERM are made in the IRM and exported (Figure 1).

Translocation mechanisms must therefore be at work all along the AM fungal colony in order to provide C to the extraradical hyphae. These mechanisms must be well regulated in order to ensure adequate supply of carbon to a mycelium that extends many centimeters, perhaps meters (Smith and Read, 1997) away from the host. To make the regulation of translocation more complex, nutrients acquired from

the soil are simultaneously translocated in the opposite direction (i.e., ERM to IRM), by undetermined processes (Bago et al., 2001).

Storage lipid translocation in AM fungi

To get a better insight into the mechanisms involved in the translocation of storage lipids along the AM fungal colony we used fluorescent labeling and *in vivo* multiphoton microscopy (Denk et al., 1990, 1995; Williams et al., 1994; Xu and Webb, 1996; Xu et al., 1996). Taking advantage of the transparent medium, convenient geometry and absence of other microorganisms in AM monoxenic cultures (Bécard and Fortin, 1988; St Arnaud et al., 1996), this approach was previously used to study nuclear behavior in symbiotic and asymbiotic AM fungal hyphae (Bago et al., 1998c, 1999a).

We have therefore followed storage lipid bodies moving in the ERM of AM fungi *Glomus intraradices* and *Gigaspora margarita* using monoxenic cultures of these two fungi with carrot transformed roots and *in vivo* multiphoton microscopy (Bago et al. 2002). Lipid bodies (also called 'oleosomes', Murphy, 1991) were labeled with the neutral lipid-specific fluorochrome Nile Red (Greenspan et al., 1985). Serial optical images ('z-series') were taken through the living tissue, and by using 3D reconstruction software, volumetric measurements of the lipid bodies were carried out (Figure 2a, b). Images at consecutive time points (time series, 't-series') of the oleosome movement along specific sites of runner hyphae were also obtained. Details on fluorochrome staining procedure, as well as on the two-photon microscope setup (i.e., excitation and emission wavelengths) are provided in Bago et al. (2002). Taken together, these data allow an estimate total TAG fluxes along symbiotic AM extraradical hyphae to be made (Bago et al., 2002).

In *G. intraradices* extraradical hyphae the volume of hyphae occupied by lipid globules ranged from 18 to 24% in zones close to the root, i.e., closer to the carbon source. When moving from these zones to the ERM growing front the amount of lipid droplets clearly decreased, to reach its minimum value in zones closer to the hypal apices. In these apical zones the volume of hypha occupied by oleosomes was only 0.5%. This gradient in lipid bodies was specially remarkable at the 'branched absorbing structures' (BAS) level. Since BAS seem to be specially active structures (Bago, 2000; Bago et al., 1998b), as are indeed all apical zones of a fungal colony, we concluded that the observed gradient in lipid bodies along the ERM is due to the progressively higher consumption of C at those apical zones. Observation of oleosome trafficking in *Gi. margarita* extraradical hyphae (Figure 2c–e) showed that in zones closer to the root lipid bodies occupy 35–50% of the total hyphal volume (Figure 2c). Similarly to *G. intraradices*, a gradient in storage lipid bodies was observed for *Gi. margarita* when moving towards the ERM growing front (Figure 2d), almost no oleosomes were seen at hyphal tip level (Figure 2e).

Time series of oleosome movement along different sites of the ERM of both *G. intraradices* and *Gi. margarita* (Bago et al., 2002) revealed that very large quantities of storage lipid bodies move along the so called 'runner hyphae' (hyphae acting as conduits for nutrient translocation and for extending the AM fungal colony radially away from the host; Bago et al., 1998a; Friese and Allen 1991). Most of the movement of such lipid bodies appeared to be carried out *via* cytoplasmic streaming, although on some occasions movement against this massive flow was observed, suggesting the possible implication of cytoskeletal elements in controlling lipid translocation along the ERM. Interestingly, oleosome movement was found to be bidirectional; and this is in agreement with labeling data, which indicate that some storage lipid indeed recirculates along the whole fungal colony and returns to intraradical fungal structures (Shachar-Hill et al., unpublished).

Using both lipid volumetric measurements and the speed with which oleosomes are transported along the runner hyphae (measured in the t-series obtained), we have estimated that mass storage lipid flow in *G. intraradices* and *Gi. margarita* is up to 0.26 and 1.34 μg/h, respectively. These data could suggest at a first glance that *Gi. margarita* is a more C-demanding fungus to the root than *G. intraradices*; however, caution should be used before reaching any further conclusion at the respect, since intraradical colonization features (e.g., number of hyphae per colonized root, total root colonization, *Arum* vs. *Paris* type mycorrhiza) should be considered first.

Global nutrient fluxes in AM fungi

Taking into account the carbon flows described above, it is possible to integrate the different macronutrient fluxes taking place in the symbiotic AM fungal colony. This scheme for C, N and P handling is shown in Fig-

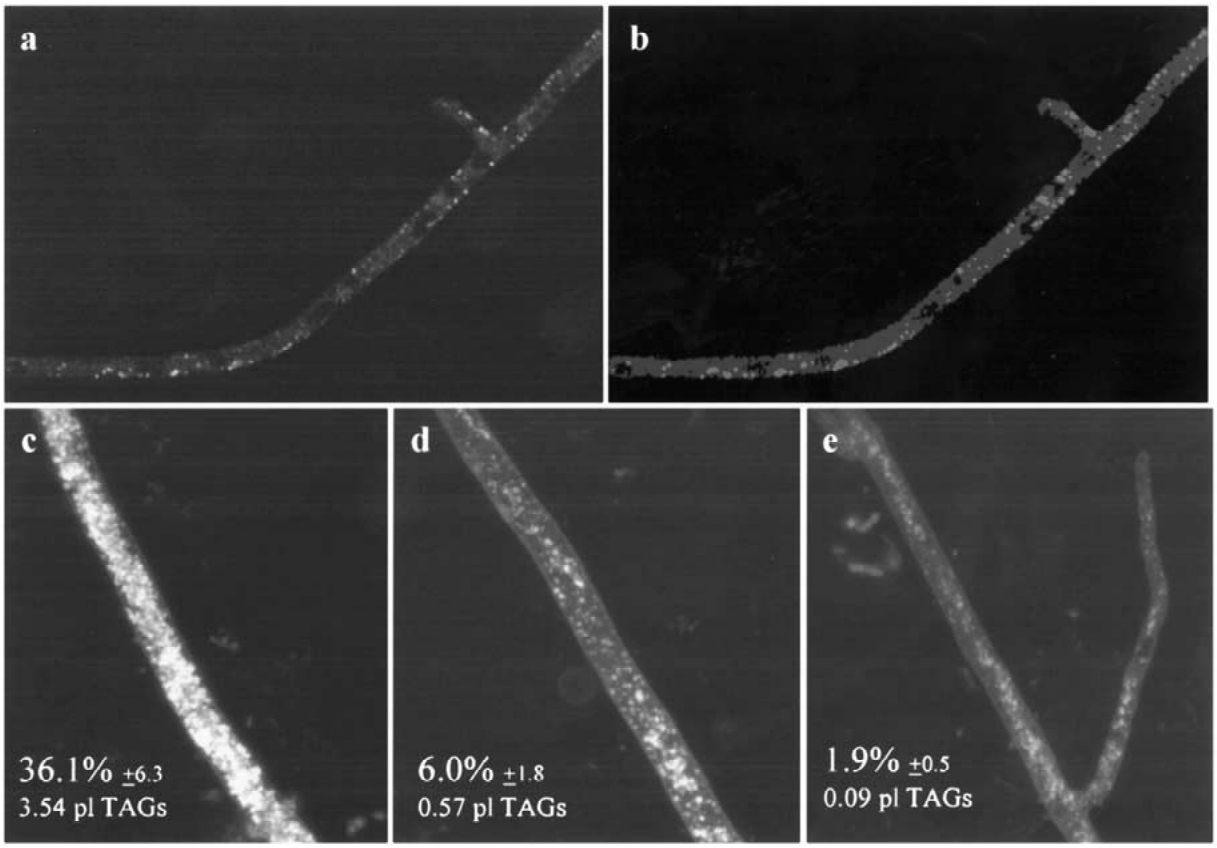

Figure 2. In vivo multiphoton microscopy of storage lipid bodies in extraradical hyphae of the AM fungus *Gigaspora margarita*. (a) 3D reconstruction of a z-series at a given point of a runner hyphae, and (b) the same z-series showing discrimination between lipid bodies (orange color) and cytoplasm (green color) as visualized after applying a software for volumetric measurement of oleosomes. (c–e) z-Series of different sites of a runner hyphae, from zones closer to the root (c), further away from the root (d) and near the hyphal growing front (e). In each micrograph the bottom-right hand side of the image is closer to the root, whereas the top-left hand side is closer to the apex. The percentage of the hyphal volume occupied by lipid droplets in each section is indicated, as well as an estimate of the total triacylglyceride (TAG) content at each site.

ure 3. This model unifies those presented recently for C fluxes (Bago et al., 2000) and postulated for soil nutrient fluxes (Bago et al., 2001), and updates the one recently presented by Pfeffer and co-workers (2001).

According to this scheme (Figure 3) profound metabolic and developmental changes in AM fungi are activated during the establishment of symbiosis with a host root. The signaling and regulation underlying these changes remain unknown, but one of the most crucial changes is probably the differenciation of the fungal partner in two metabolically different but interconnected parts: the intraradical and the extraradical mycelium, with the former being the only location for hexose acquisition. Such hexose fuels metabolism and growth of the IRM, and it is transformed into typical fungal molecules such as trehalose, glycogen and chitin. In order to fuel extraradical fungal development, glycogen is translocated (Figure 3, '1') along newly developed runner hyphae, which would extend the extraradical fungal colony within the soil. Oleosomes containing TAGs are also shipped from the IRM (Figure 3, '2'), some of them recirculating throughout the fungal colony (note the bidirectional arrow of translocation event '2'). These lipid bodies also play key roles in fungal morphogenic and reproductive events such as sporulation.

Extraradical hyphae developing within the substrate take up soil nutrients (mostly P, but also N), and by means of mechanisms that are as yet a matter of inference and postulate (Smith and Read, 1997; Bago et al., 2001) are translocated to the intraradical phase of the fungal colony (Figure 3, '3'), and finally released to the host root. It is possible that amino acids such as Arg are involved in *N*-translocation. In that case,

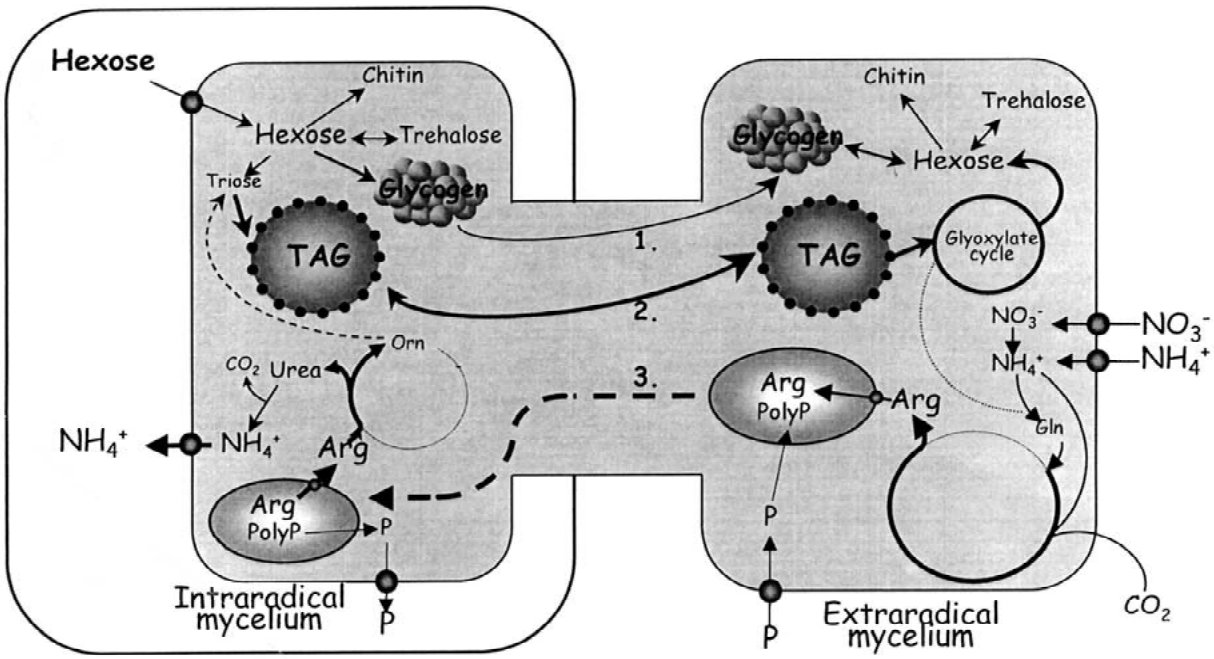

Figure 3. Updated general overview of the translocation events implicating the three major nutrients (C, P and N) which may take place along the AM fungal colony.

carbon and nitrogen translocation along AM fungal hyphae would be linked through the amino acid carbon skeleton. Moreover, since this translocation might be also linked to polyP translocation, as it is the case in ectomycorrhizas (Bütehorn et al., 1999) it could result in that translocation of the three major chemical elements (C, N, P) in AM fungi would be linked. Once released within intraradical fungal structures such carbon skeletons would re-join the C intraradical pool and be used according to fungal needs either for metabolic, translocation or structural purposes.

This working model is consistent with a large number of studies on the AM symbioses, nevertheless it postulates a number of completely untested mechanisms and invites scrutiny and study by a variety of methods.

Possible mechanisms for regulating nutrient translocation in AM fungi

When considering the scheme presented in Figure 3, we might realize that sophisticated translocation processes occur along long hyphal distances that require regulation. How might a coenocytic 'lower' fungus exert control over large bi-directional nutrient flows over long distances?

AM hyphae (both intra- and extraradical) are coenocytic and multinuclear (Balestrini et al., 1992) and these nuclei are distributed very regularly within extraradical hyphae (Figure 4; Bago et al., 1999a). Nuclei are located approximately every 36.0 μm and particular nuclei have been postulated to control particular cellular events (e.g., branching events; Bago et al., 1999a). It is known that transcript levels for beta tubulin vary during the fungal life cycle (Requena et al., 2000) and it may be that at the gene expression level, regulating translocation along the coenocytic AM fungal colony involves an even finer spatial pattern of transcriptional control, with different nuclei in the colony having different transcription profiles.

Nuclear migration is required to distribute nuclei through the hyphal ERM, and this process has been studied using multiphoton microscopy, together with *in vivo* DAPI staining (Figure 4; Bago et al., 1999a). Such migration is bi-directional, apparently independent of the cytoplasmic streaming. These observations put forward once more the probably fundamental role cytoskeletal elements play in the organization and functioning of the AM fungal colony. Microscopy and immunological identification has shed some light on these key components of the AM fungal colony (Ström et al., 1994; Timonen et al., 2001), and the

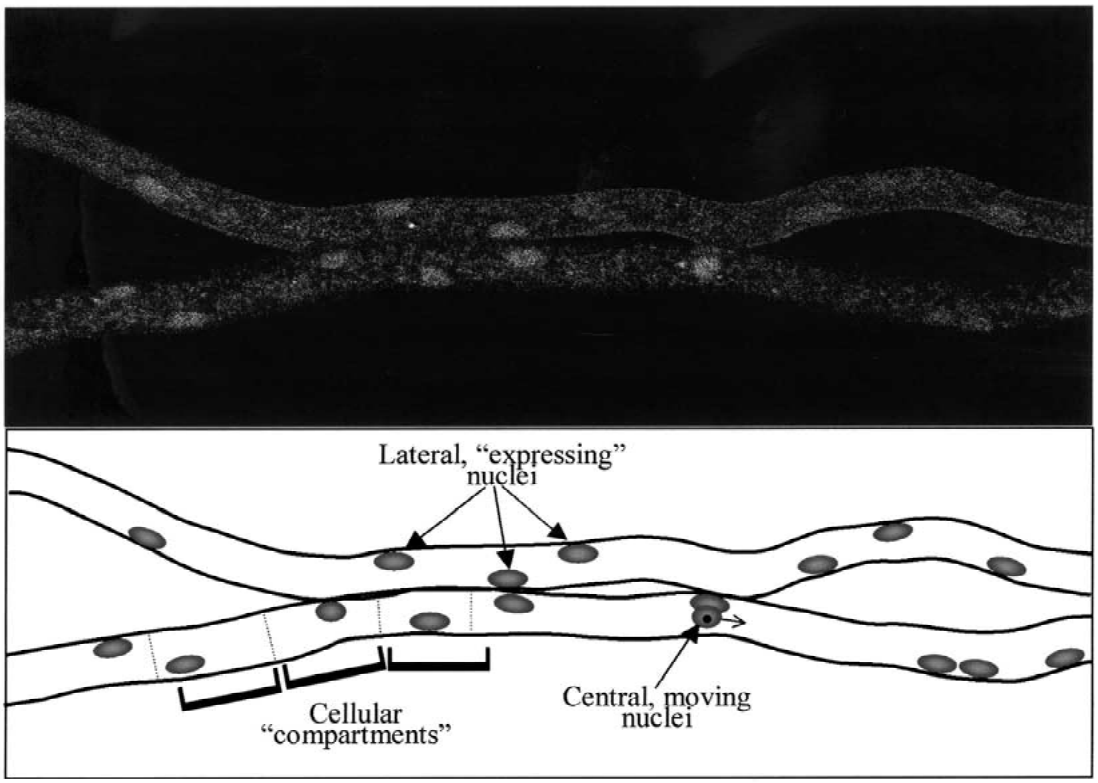

Figure 4. In vivo multiphoton microscopy of fungal nuclei along an extraradical hypha of *Glomus intraradices* (upper pannel) and interpretation of the micrograph (lower pannel), showing the main features of nuclear distribution along ERM in this fungus.

identification of sequences for several key cytoskeletal proteins is described in Jun et al. (2002). Clearly more research is needed to understand translocation processes in AM fungi, and not only as they relate to nutrient distribution along hyphae, but also concerning cellular organelle positioning, and to the functioning of the AM fungal colony.

References

Aström H, Giovannetti M and Raudaskoski M 1994 Cytoskeletal components in the arbuscular mycorrhizal fungus *Glomus mosseae*. MPMI 7, 309–312.

Bago, B 2000 Putative sites for nutrient uptake in arbuscular mycorrhizal fungi. Plant Soil 226, 263–274.

Bago B, Azcón-Aguilar C and Piché Y 1998a Architecture and developmental dynamics of the external mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown under monoxenic conditions. Mycologia 90, 52–62.

Bago B, Azcón-Aguilar C, Goulet A and Piché Y 1998b Branched absorbing structures (BAS): a feature of the extraradical mycelium of symbiotic arbuscular mycorrhizal fungi. New Phytol. 139, 375–388.

Bago B, Zipfel W, Williams RC and Piché Y 1999a Nuclei of symbiotic arbuscular mycorrhizal fungi as revealed by in vivo two-photon microscopy. Protoplasma 209, 77–89.

Bago B, Pfeffer PE, Douds DD Jr, Brouillette J, Bécard G and Shachar-Hill Y 1999b Carbon metabolism in arbuscular mycorrhizal spores as revealed by NMR spectroscopy. Plant Physiol. 121, 263–271.

Bago B, Zipfel W, Williams RC, Chamberland H, Lafontaine J-G, Webb WW and Piché Y 1998c *In vivo* studies on the nuclear behavior of the arbuscular mycorrhizal fungus *Gigaspora rosea* grown under axenic conditions. Protoplasma 203, 1–15.

Bago B, Pfeffer PE and Shachar-Hill Y 2000 Carbon metabolism and transport in arbuscular mycorrhizas. Plant Physiol. 124, 949–957.

Bago B, Pfeffer PE and Shachar-Hill Y 2001 Could the urea cycle be translocating nitrogen in arbuscular mycorrhizal fungi? New Phytol. 149, 4–8.

Bago B, Zipfel W, Williams RC, Jun J, Arreola R, Pfeffer PE, Lammers PJ and Shachar-Hill Y 2002 Translocation and utilization of fungal lipid in the arbuscular mycorrhizal symbiosis. Plant Physiol 128, 108–124.

Balestrini R, Bianciotto V and Bonfante-Fasolo P 1992 Nuclear architecture and DNA location in two VAM fungi. Mycorrhiza 1, 105–112.

Bécard G and Fortin A 1988 Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. New Phytol. 108, 211–218.

Beilby JP 1983 Effects of inhibitors on early protein, RNA, and lipid synthesis in germinating vesicular-arbuscular mycorrhizal fungal spores of *Glomus caledonium*. Can. J. Bot. 29, 596–601.

Beilby JP and Kidby DK 1980 Biochemistry of ungerminated and germinated spores of the vesicular-arbuscular mycorrhizal fungus *Glomus caledonium*: changes in neutral and polar lipids. J. Lipid Res. 21, 739–750.

Burleigh S H and Ellegaard Bechman I 2002 Plant nutrient transporter regulation in arbuscular mycorrhiza. Plant Soil 244, 247–251.

Bütehorn B, Gianinazzi-Pearson V and Franken P 1999 Quantification of beta-tubulin RNA expression during asymbiotic and symbiotic development of the arbuscular mycorrhizal fungus *Glomus mosseae*. Mycol. Res. 103, 360–364.

Cooper KM and Lösel DM 1978 Lipid physiology of vesicular arbuscular mycorrhiza. New Phytol. 80, 143–151.

Denk W, Strickler JH and Webb WW 1990 Two-photon laser scanning fluorescence microscopy. Science 248, 73–76.

Denk W, Piston DN and Webb WW 1995 Two-photon molecular excitation in laser scanning fluorescence microscopy. *In* Handbook of Biological Confocal Microscopy. Ed. JB Pawley. pp 445–458. Plenum Pres, New York.

Ferrol N, Barea J M and Azcón-Aguilar 2002 Mechanisms of nutrient transport across interfaces in arbuscular mycorrhizas. Plant Soil 244, 231–237.

Friese CF and MF Allen 1991 The spread of VA mycorrhizal fungal hyphae in the soil: inoculum types and external hyphal architecture. Mycologia 83, 409–418.

Gaspar ML, Pollero RJ and Cabello MN 1994 Triacylglycerol consumption during spore germination of vesicular-arbuscular mycorrhizal fungi. JAOCS 71, 449–452.

Gaspar L, Pollero R and Cabello M 1997 Partial purification and characterization of a lipolytic enzyme from spores of the arbuscular mycorrhizal fungus *Glomus versiforme.* Mycologia 89, 610–614.

George E, Marschner H and Jakobsen I 1995 Role of arbuscular-mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. Crit. Rev. Biotechnol. 15, 257–270.

Gianinazzi-Pearson V, Smith SE, Gianinazzi S and Smith FA 1991 Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhizas. V. Is H^+-ATPase a component of ATP-hydrolysing enzyme activities in plant-fungus interfaces? New Phytol. 117, 61–74.

Gow NAR and Gadd GM 1984 The Growing Fungus. Chapman & Hall, London, UK. 472 pp.

Greenspan P, Mayer EP and Fowler SD 1985 Nile Red: a selective fluorescent stain for intracellular lipid droplets. J Cell Biol 100, 965–973.

Harrier LA, Wright F and Hooker JE 1998 Isolation of the 3-phosphoglycerate kinase gene of the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe. Curr. Genet. 34, 386–392.

Ho I, Trappe JM 1973 Translocation of ^{14}C from *Festuca* plants to their endomycorrhizal fungi. Nature 244, 30–31.

Jabaji-Hare S 1988 Lipid and fatty acid profiles of some vesicular-arbuscular mycorrhizal fungi: contribution to taxonomy. Mycologia 80, 622–629.

Jakobsen I 1999 Transport of phosphorus and carbon in VA mycorrhizas. *In* Mycorrhiza: Structure, Function, Molecular Biology and Biotechnology, 2nd Edition. Eds. A Varma, B Hock. pp. 305–332. Springer, Berlin.

Jennings DH 1995 The Physiology of Fungal Nutrition. Cambridge University Press, Cambridge.

Jun J, Abubaker J, Rehrer C, Pfeffer P E, Schachar-Hill Y and Lammers P J 2002 Expression in an arbuscular mycorrhizal fungus of genes putatively involved in metabolism, transport, the cyto skeleton and the cell cycle. Plant Soil 244, 141–148.

Koide RT and Schreiner RP 1992 Regulation of the vesicular-arbuscular mycorrhizal symbiosis. Annu. Rev. Plant. Physiol. Mol. Biol. 43, 557–581.

Lammers P, Jun J, Abubaker J, Arreola R, Gopalan A, Bago B, Hernández C, Allen J, Douds DD, Pfeffer PE and Shachar-Hill Y 2001 Gene expression and glyoxylate cycle activity during of an arbuscular mycorrhizal fungus. Plant Physiol. 127, 1287–1298.

Lösel DM and Cooper KM 1979 Incorporation of ^{14}C-labelled substrates by uninfected and VA mycorrhizal roots of onions. New Phytol. 834.

Martin F, Canet D and Marchal P 1985 ^{13}C NMR study of the mannitol cycle and trehalose synthesis during glucose utilization by ectomycorrhizal ascomycete *Cenococcum graniforme*. Plant Physiol. 77, 449–502.

Martin F, Boiffin V and Pfeffer PE 1998 Carbohydrate and amino acid metabolism in the *Eucalyptus globulus–Pisolithus tinctorius* ectomycorrhiza during glucose utilization. Plant Physiol. 118, 627–635.

MacDonald RM and Lewis M 1978 The occurrence of some acid phosphatases and dehydrogenases in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. New Phytol. 80, 135–141.

Murphy DJ 1991 Storage lipid bodies in plants and other organisms. Prog. Lipid Res. 29, 299–324.

Nagy S, Nordby HE and Nemec S 1980 Composition of lipids in roots of six citrus cultivars infected with the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. New Phytol. 85, 377–382.

Pfeffer PE and Shachar-Hill Y 1996 Plant/microbe symbioses. *In* Nuclear Magnetic Resonance in Plant Biology. Eds. Y Shachar-Hill and PE Pfeffer. pp. 77–107. APS Press, Rockville, MD.

Pfeffer PE, Douds DD, Bécard G and Shachar-Hill Y 1999 Carbon uptake and the metabolism and transport of lipids in an arbuscular mycorrhiza. Plant Physiol. 120, 587–598.

Pfeffer PE, Bago B and Shachar-Hill Y 2001 Exploring mycorrhizal function with NMR spectroscopy. New Phytol. 150, 548–554.

Requena N, Mann P and Franken P 2000 A homologue of the cell cycle check point TOR2 from *Saccharomyces cerevisiae* exists in the arbuscular mycorrrhizal fungus *Glomus mosseae*. Protoplasma 212, 89–98.

Saito M 1995 Enzyme activities of the internal hyphae and germinated spores of an arbuscular mycorrhizal fungus, *Gigaspora margarita* Becker & Hall. New Phytol. 129, 425–431.

Séjalon-Delmas N, Rogister I, Jauneau A, Roux C and Bécard G 2001 Carbon uptake during symbiotic, asymbiotic and presymbiotic stages of the AM fungus Glomus intraradices. Proceedings of ICOM3, Adelaide, p. P2 137.

Shachar-Hill Y, Pfeffer PE, Douds D, Osman SF, Doner LW and Ratcliffe RG 1995 Partitioning of intermediate carbon metabolism in VAM colonized leek. Plant Physiol. 108, 7–15.

Smith SE, Read DJ 1997 Mycorrhizal Symbiosis. Academic Press, London. 605 pp.

Smith SE and Smith FA 1990 Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. New Phytol. 114, 1–38.

Smith FA and Smith SE 1997 Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. New Phytol. 137, 373–388.

Solaiman MD and Saito M 1997 Use of sugars by intraradical hyphae of arbuscular mycorrhizal fungi revealed by radiorespirometry. New Phytol. 136, 533–538.

St-Arnaud M, Hamel C, Vimard B, Caron M and Fortin JA 1996 Enhanced hyphal growth and spore production of the arbuscular

mycorrhizal fungus *G. intraradices* in an *in vitro* system in the absence of host roots. Mycol. Res. 100, 328–332.

Timonen S, Smith FA and Smith SE 2001 Microtubules of mycorrhizal fungus *Glomus intraradices* in symbiosis with tomato roots. Can. J. Bot. 79, 307–313.

Williams RM, Piston DW and Webb WW 1994 Two-photon molecular excitation provides intrinsic 3-dimensional resolution for laser-based microscopy and microphotochemistry. FASEB J. 8, 804–813.

Xu C and Webb WW 1996 Measurement of two-photon excitation cross sections of molecular fluorophores with data from 690 to 1050 nm. J. Opt. Soc. Am. (B) 13, 481–491.

Xu C, Zipfel W, Shear JB, Williams RM and Webb WW 1996 Multiphoton fluorescence excitation: New spectral windows for biological non-linear microscopy. Proc. Natl. Acad. Sci. USA 93, 10763–10768.